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DUPONT HASKELL GLOBAL CENTERS FOR HEALTH & ENVIRONMENTAL SCIENCES Discovery Toxicology Group

Biopersistence and Pharmacokinetic Screen in the Rat

WORK REQUEST: 17473 SERVICE CODE: 415 HASKELL NUMBER: 28307 DUPONT REPORT NUMBER: 24286 TESTING SOP NUMBER: BT004-T-002 STUDY START DATE: 2-October-2007 STUDY END DATE: 10-October-2007 NOTEBOOK(s): E-104276-CZ

WORK BY: Critical Path Services STUDY DIRECTOR: Shawn A. Gannon, Senior Staff Toxicologist REPORT ISSUE DATE: 13-February-2008

STUDY DESIGN

Test Substance:	HFPO Dimer Acid
Lot/Batch Number:	E112820-46MC
Purity:	98%
Species:	Rat
Strain:	Crl:CD(SD)
Vendor:	Charles River Laboratories, Raleigh, North Carolina, U.S.A.
Sex:	Male and Female
Route:	Oral
Age at Study Start:	7-12 weeks
Total Group Size:	3/sex/dose level
Dose Frequency:	Single dose
Dose Vehicle:	Water
Dose:	Low dose 10 mg/kg, high dose 30 mg/kg
Dose Volume:	4 mL/kg
Blood Sample Time Points:	0, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hours

OBJECTIVE

To generate preliminary pharmacokinetic data. Fat and liver were analyzed for parent compound to provide an estimate of tissue:plasma ratio.

METHODS

LC/MS Sample Analyses

A. Plasma Samples

Plasma Samples - The plasma samples were received and stored frozen prior to laboratory use. The samples were prepared for analysis by pipeting 150 μ L acetonitrile into a 1.7 mL microcentrifuge tube, and pipeting 50.0 μ L of plasma sample. The sample tubes were then vortexed for 1 minute and centrifuged at 14,000 RCF for 30 minutes. After centrifugation, 100 μ L of sample supernatant was placed into a HPLC vial and 400 μ L of HPLC grade water was added and mixed. As necessary, additional sample dilutions were performed using the 15% acetonitrile in HPLC grade water solvent to ensure that the sample responses were within the calibration curve.

B. Liver and Fat Samples

The liver and fat samples were received and stored frozen prior to laboratory use. The liver tissue samples were extracted in Acetonitrile with 4% perchloric acid while the fat samples were extracted in isopropyl alcohol. The calibration standards were prepared in the appropriate matched solvents.

1. Fat and Liver Extract Preparation.

Fat and Liver Extract Preparation. The fat and liver samples were preprocessed by chopping the tissue samples into small pieces and weighing (0.5 grams) into disposable 15-mL polypropylene centrifuge tubes. Five 5/32" ball bearings were added to each tube, and a pipet was used to add 5000 µL of the appropriate extraction solvent. The tubes were sealed with parafilm, and inserted into a SPEX Certiprep Genogrinder and homogenized for 4 minutes at 1400 strokes/minute. After homogenization, the tubes were centrifuge at 4125 rpm for 20 min at room temperature. The extract supernatant was transferred into glass vials and frozen prior to further sample preparation.

2. Fat and Liver Extract Analysis.

Approximately 100 mg of Envi-Carb graphitized carbon sorbent was placed into a 1.7-mL microcentrifuge tube. A pipet was used to add 50.0 μ L of glacial acetic acid directly to the sorbent. Next, a pipet was used to add 1000 μ L of sample extract into the centrifuge tube. The tubes were capped, vortexed briefly, and then centrifuged at 10,000 RCF for 10 minutes at room temperature. After centrifugation, a pipet was used to add 525 μ L of the 1.7-mL microcentrifuge tube supernatant into a new 1.7 mL microcentrifuge tube and 475 μ L of HPLC grade water and mixed. The samples were centrifuged at 10,000 RCF for 5 minutes at room temperature, and supernatant transferred into HPLC vials for analysis. As necessary, additional sample dilutions were performed using the matched solvent to ensure that the sample responses were within the calibration curve.

The prepared samples were analyzed by LC/MS using the following parameters:

HPLC Instrument: MS Instrument:	Agilent Model 1200 Applied Biosystems API 4000								
<i>LC Parameters:</i> Column: Mobile Phase:	Zorbax RX-C8; 150 x 2.1 mm with 5 μ particle size A: 0.15% acetic acid and 0.15% triethyl amine in HPLC grade water B: 0.15% acetic acid and 0.15% triethyl amine in acetonitrile								
Column Temperature: Injection Volume:	35°C 10.0 μL								
MS Parameters:									
Ion Source:	Turbo Spray,	Negative Ior	1						
Temperature (TEM):	450								
Dwell	300 msec								
Curtain Gas Flow (CUR):	50.0								
GS1:	11								
GS2:	70								
IonSpray (IS) Voltage:	-4500								
CAD	10.0								
EP	-10.0	•							
Quadrupole Resolution:	Quad. 1: Uni	1t							
MDM Cottings	Quad. 3: Uni	02 Mass	חח	CE	CVD				
WRW Settings	329.00	285.00	-20	-10	-5				
HPLC Gradient	Total Time	Flow Rate							
	(min)	(mL/min)	A(%)	B(%)					
	0.00	250	60.0	40.0					
	5.00	250	60.0	40.0					
	5.01	350	10.0	90.0					
	9.00	350	10.0	90.0					
	9.01	350	60.0	40.0					
	19.00	350	60.0	40.0					
	19.01	250	60.0	40.0					
	20.00	250	60.0	40.0					

RESULTS

The individual plasma sample results are provided in Appendix A The individual liver sample results are provided in Appendix B The individual fat sample results are provided in Appendix C

Plasma

Graph:



Tissue/p	lasma	ratio	at	sacrif	ice	•
						-

Fat:	All fat samples below LOQ for males and females
Liver:	10 mg/kg male = 0.64; 30 mg/kg male = 0.71
Comments:	The tissue LOQ was approximately 20 ng/g. All female liver concentration values
	<loq.< td=""></loq.<>

Appendix A Individual LC/MS Plasma Sample Results

10 mg/kg results

	H-28307 Plasma Concentration for the specified timepoint (ng/mL)														
Rat		15	30	1	2	4	8	12	24	48	72	96	120	144	168
Number	Predose	min	min	Hour	Hour	Hour	Hour	Hour	Hour	Hour	Hour	Hour	Hour	Hour	Hour
D . #4		22 000	25200	64000	F O 600	22.400	0.400	4.500		0.6.0	0 0 4				20.2
Rat #1	<loq< td=""><td>22000</td><td>35200</td><td>64800</td><td>50600</td><td>32400</td><td>8480</td><td>4580</td><td>754</td><td>86.8</td><td>92.4</td><td>54</td><td>67.4</td><td>35.2</td><td>30.2</td></loq<>	22000	35200	64800	50600	32400	8480	4580	754	86.8	92.4	54	67.4	35.2	30.2
Rat #2	<loq< td=""><td>34600</td><td>51200</td><td>76000</td><td>56600</td><td>35800</td><td>10820</td><td>5360</td><td>1776</td><td>161</td><td>94.8</td><td>47.4</td><td>42</td><td>55.8</td><td>43.2</td></loq<>	34600	51200	76000	56600	35800	10820	5360	1776	161	94.8	47.4	42	55.8	43.2
Rat #3	<loq< td=""><td>12400</td><td>42000</td><td>68600</td><td>63000</td><td>50400</td><td>10700</td><td>5460</td><td>1250</td><td>156</td><td>59</td><td>69.6</td><td>48.4</td><td>28</td><td>49.8</td></loq<>	12400	42000	68600	63000	50400	10700	5460	1250	156	59	69.6	48.4	28	49.8
Rat #4	<loq< td=""><td>37400</td><td>35200</td><td>12920</td><td>3200</td><td>626</td><td>77.6</td><td>39.6</td><td>39.4</td><td>35.2</td><td>34.4</td><td>30.2</td><td><loq< td=""><td><loq< td=""><td><loq< td=""></loq<></td></loq<></td></loq<></td></loq<>	37400	35200	12920	3200	626	77.6	39.6	39.4	35.2	34.4	30.2	<loq< td=""><td><loq< td=""><td><loq< td=""></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""></loq<></td></loq<>	<loq< td=""></loq<>
Rat #5	<loq< td=""><td>33800</td><td>21600</td><td>11800</td><td>5180</td><td>580</td><td>532</td><td>81</td><td><loq< td=""><td><loq< td=""><td>26.2</td><td><loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""></loq<></td></loq<></td></loq<></td></loq<></td></loq<></td></loq<></td></loq<>	33800	21600	11800	5180	580	532	81	<loq< td=""><td><loq< td=""><td>26.2</td><td><loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""></loq<></td></loq<></td></loq<></td></loq<></td></loq<></td></loq<>	<loq< td=""><td>26.2</td><td><loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""></loq<></td></loq<></td></loq<></td></loq<></td></loq<>	26.2	<loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""></loq<></td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td><loq< td=""></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""></loq<></td></loq<>	<loq< td=""></loq<>
Rat #6	<loq< td=""><td>31200</td><td>23800</td><td>15980</td><td>8080</td><td>4000</td><td>734</td><td>112</td><td><loq< td=""><td>26</td><td><loq< td=""><td>37</td><td><loq< td=""><td><loq< td=""><td><loq< td=""></loq<></td></loq<></td></loq<></td></loq<></td></loq<></td></loq<>	31200	23800	15980	8080	4000	734	112	<loq< td=""><td>26</td><td><loq< td=""><td>37</td><td><loq< td=""><td><loq< td=""><td><loq< td=""></loq<></td></loq<></td></loq<></td></loq<></td></loq<>	26	<loq< td=""><td>37</td><td><loq< td=""><td><loq< td=""><td><loq< td=""></loq<></td></loq<></td></loq<></td></loq<>	37	<loq< td=""><td><loq< td=""><td><loq< td=""></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""></loq<></td></loq<>	<loq< td=""></loq<>

30 mg/kg results

	H-28307 Plasma Concentration for the specified timepoint (ng/mL)														
Rat		15	30	1	2	4	8	12	24	48	72	96	120	144	168
Number	Predose	min	min	Hour	Hour	Hour	Hour	Hour	Hour	Hour	Hour	Hour	Hour	Hour	Hour
Rat #1	<loq< td=""><td>73800</td><td>97800</td><td>128800</td><td>163200</td><td>64800</td><td>28400</td><td>9920</td><td>1684</td><td>330</td><td>472</td><td>324</td><td>165</td><td>138</td><td>102</td></loq<>	73800	97800	128800	163200	64800	28400	9920	1684	330	472	324	165	138	102
Rat #2	<loq< td=""><td>61000</td><td>107600</td><td>135200</td><td>169600</td><td>124800</td><td>21000</td><td>8860</td><td>1412</td><td>580</td><td>444</td><td>254</td><td>186</td><td>174</td><td>143</td></loq<>	61000	107600	135200	169600	124800	21000	8860	1412	580	444	254	186	174	143
Rat #3	<loq< td=""><td>92400</td><td>110000</td><td>230400</td><td>70000</td><td>58000</td><td>34000</td><td>6780</td><td>1530</td><td>612</td><td>360</td><td>330</td><td>180</td><td>158</td><td>140</td></loq<>	92400	110000	230400	70000	58000	34000	6780	1530	612	360	330	180	158	140
Rat #4	<loq< td=""><td>96800</td><td>92000</td><td>42800</td><td>7500</td><td>1320</td><td>530</td><td>864</td><td>11.5</td><td>26.4</td><td>46.6</td><td><loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""></loq<></td></loq<></td></loq<></td></loq<></td></loq<>	96800	92000	42800	7500	1320	530	864	11.5	26.4	46.6	<loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""></loq<></td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td><loq< td=""></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""></loq<></td></loq<>	<loq< td=""></loq<>
Rat #5	<loq< td=""><td>107600</td><td>92400</td><td>42800</td><td>18580</td><td>1204</td><td>640</td><td>578</td><td>21.4</td><td><loq< td=""><td>102</td><td>24.8</td><td>23</td><td><loq< td=""><td><loq< td=""></loq<></td></loq<></td></loq<></td></loq<>	107600	92400	42800	18580	1204	640	578	21.4	<loq< td=""><td>102</td><td>24.8</td><td>23</td><td><loq< td=""><td><loq< td=""></loq<></td></loq<></td></loq<>	102	24.8	23	<loq< td=""><td><loq< td=""></loq<></td></loq<>	<loq< td=""></loq<>
Rat #6	<loq< td=""><td>78800</td><td>63600</td><td>62000</td><td>21400</td><td>1196</td><td>242</td><td>176</td><td>29.2</td><td>50</td><td><loq< td=""><td>23</td><td><loq< td=""><td><loq< td=""><td><loq< td=""></loq<></td></loq<></td></loq<></td></loq<></td></loq<>	78800	63600	62000	21400	1196	242	176	29.2	50	<loq< td=""><td>23</td><td><loq< td=""><td><loq< td=""><td><loq< td=""></loq<></td></loq<></td></loq<></td></loq<>	23	<loq< td=""><td><loq< td=""><td><loq< td=""></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""></loq<></td></loq<>	<loq< td=""></loq<>

Note: Rats 1-3 are male, 4-6 are female.

		Liver Conc (ng/g)	Plasma Conc (ng/mL)	T:P ratio	Average T:P ratio	S.D. T:P ratio
Male	10 mg/kg Rat #1	30.6	30.2	1.01		
	10 mg/kg Rat #2	20	43.2	0.46		
	10 mg/kg Rat #3	21.5	49.8	0.43	0.64	0.33
Female	10 mg/kg Rat #4	<loq< td=""><td><loq< td=""><td>NA</td><td></td><td></td></loq<></td></loq<>	<loq< td=""><td>NA</td><td></td><td></td></loq<>	NA		
	10 mg/kg Rat #5	<loq< td=""><td><loq< td=""><td>NA</td><td></td><td></td></loq<></td></loq<>	<loq< td=""><td>NA</td><td></td><td></td></loq<>	NA		
	10 mg/kg Rat #6	<loq< td=""><td><loq< td=""><td>NA</td><td>NA</td><td>NA</td></loq<></td></loq<>	<loq< td=""><td>NA</td><td>NA</td><td>NA</td></loq<>	NA	NA	NA
Male	30 mg/kg Rat #1	90.6	102	0.89		
	30 mg/kg Rat #2	91.2	143	0.64		
	30 mg/kg Rat #3	84.7	140	0.61	0.71	0.15
Female	30 mg/kg Rat #4	<loq< td=""><td><loq< td=""><td>NA</td><td></td><td></td></loq<></td></loq<>	<loq< td=""><td>NA</td><td></td><td></td></loq<>	NA		
	30 mg/kg Rat #5	<loq< td=""><td><loq< td=""><td>NA</td><td></td><td></td></loq<></td></loq<>	<loq< td=""><td>NA</td><td></td><td></td></loq<>	NA		
	30 mg/kg Rat #6	<loq< td=""><td><loq< td=""><td>NA</td><td>NA</td><td>NA</td></loq<></td></loq<>	<loq< td=""><td>NA</td><td>NA</td><td>NA</td></loq<>	NA	NA	NA

Appendix B Individual LC/MS Liver Sample Results

		Fat Conc (ng/g)	Plasma Conc (ng/mL)	T:P ratio	Average T:P ratio	S.D. T:P ratio
Male	10 mg/kg Rat #1	<l00< td=""><td>30.2</td><td>NA</td><td></td><td></td></l00<>	30.2	NA		
	10 mg/kg Rat #2	<loq< td=""><td>43.2</td><td>NA</td><td></td><td></td></loq<>	43.2	NA		
	10 mg/kg Rat #3	<loq< td=""><td>49.8</td><td>NA</td><td>NA</td><td>NA</td></loq<>	49.8	NA	NA	NA
		<loq< td=""><td></td><td></td><td></td><td></td></loq<>				
Female	10 mg/kg Rat #4	<loq< td=""><td><loq< td=""><td>NA</td><td></td><td></td></loq<></td></loq<>	<loq< td=""><td>NA</td><td></td><td></td></loq<>	NA		
	10 mg/kg Rat #5	<loq< td=""><td><loq< td=""><td>NA</td><td></td><td></td></loq<></td></loq<>	<loq< td=""><td>NA</td><td></td><td></td></loq<>	NA		
	10 mg/kg Rat #6	<loq< td=""><td><loq< td=""><td>NA</td><td>NA</td><td>NA</td></loq<></td></loq<>	<loq< td=""><td>NA</td><td>NA</td><td>NA</td></loq<>	NA	NA	NA
		<loq< td=""><td></td><td></td><td></td><td></td></loq<>				
Male	30 mg/kg Rat #1	<loq< td=""><td>102</td><td>NA</td><td></td><td></td></loq<>	102	NA		
	30 mg/kg Rat #2	<loq< td=""><td>143</td><td>NA</td><td></td><td></td></loq<>	143	NA		
	30 mg/kg Rat #3	<loq< td=""><td>140</td><td>NA</td><td>NA</td><td>NA</td></loq<>	140	NA	NA	NA
		<loq< td=""><td></td><td></td><td></td><td></td></loq<>				
Female	30 mg/kg Rat #4	<loq< td=""><td><loq< td=""><td>NA</td><td></td><td></td></loq<></td></loq<>	<loq< td=""><td>NA</td><td></td><td></td></loq<>	NA		
	30 mg/kg Rat #5	<loq< td=""><td><loq< td=""><td>NA</td><td></td><td></td></loq<></td></loq<>	<loq< td=""><td>NA</td><td></td><td></td></loq<>	NA		
	30 mg/kg Rat #6	<loq< td=""><td><loq< td=""><td>NA</td><td>NA</td><td>NA</td></loq<></td></loq<>	<loq< td=""><td>NA</td><td>NA</td><td>NA</td></loq<>	NA	NA	NA

Appendix C Individual LC/MS Fat Sample Results